Finanziato dall'Unione europea NextGenerationEU
Ministero dell'Università e della Ricerca
Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA
THE Tuscany Health Ecosystem

# Role of Simvastatin in cardioprotection: effects on Doxorubicin-induced cardiotoxicity

Roberta Vitale[1], Mariangela Mazzone[2], Maria Carmela Di Marcantonio[2], Barbara Palà[3], Gabriella Mincione[2], Stefania Marzocco[1], Ada Popolo[1].
1 Department of Pharmacy, University of Salerno, 84084 Fisciano (SA), Italy
2 Department of Innovative Technologies in Medicine and Dentistry, University "G. D'Annunzio" Chieti-Pescara, 66100 Chieti, Italy
3 Division of Cardiology, Department of Clinical and Molecular Medicine, Sant'Andrea Hospital, Sapienza University of Rome, 00189 Roma, Italy
Email: rvitale@unisa.it

42° CONGRESSO NAZIONALE DELLA SOCIETÀ ITALIANA DI FARMACOLOGIA
SCIENCE TODAY FOR A BETTER MEDICINE TOMORROW
SORRENTO (NA) 13-16 NOVEMBRE 2024

## INTRODUCTION

- Cardiotoxicity is the main side effect of Doxorubicin [1]
- Cardiomyocytes damage can occur as early as the first administration of the drug [2,3]
- Current research is focused on identifying potential drugs that can mitigate cardiac side effects without compromising Doxorubicin's anti-tumor efficacy
- Statins are commonly used as cardioprotective agents [4,5]
- Statins may influence the expression of Cx43, a protein member of the Gap Junctions (GJS) family that plays a crucial role in the early adaptative response to Doxorubicin-induced stress [6]
- Combination therapy with statins has been found to enhance the anti-tumor activity of Doxorubicin and Cyclophosphamide in breast cancer cells [7]

## AIM

The purpose of this study was to evaluate the protective effects of Simvastatin in a cellular model of Doxorubicin-induced acute cardiotoxicity.

## METHODS

Human Cardiomyocytes cell line (HCM) was treated with Simvastatin (10μM) for 4 hours and then co-exposed to Simvastatin (Sim) and Doxorubicin (Doxo) (1μM) for the next 20 hours.

## RESULTS

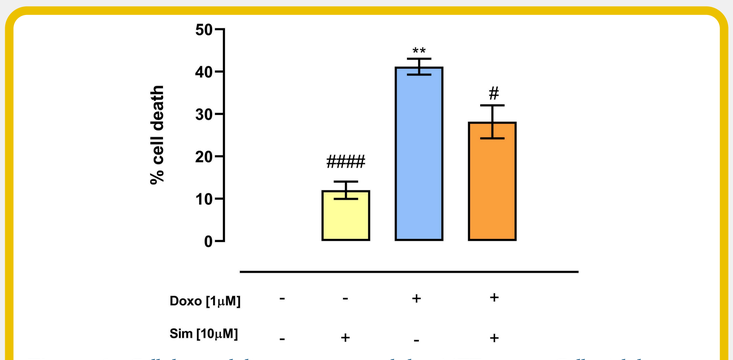


**Figure 1.** Cellular viability was assessed by MTT assay. Cell viability was calculated as % of dead cells = 100 - ([OD treated/ OD control] x 100). Data were analyzed using One-Way ANOVA followed by the Bonferroni multiple comparisons. Values are expressed as mean ± SEM of % cell death (n=3). ** p<0.01 vs control cells; # p<0.05 and #### p<0.001 vs Doxo-treated cells.

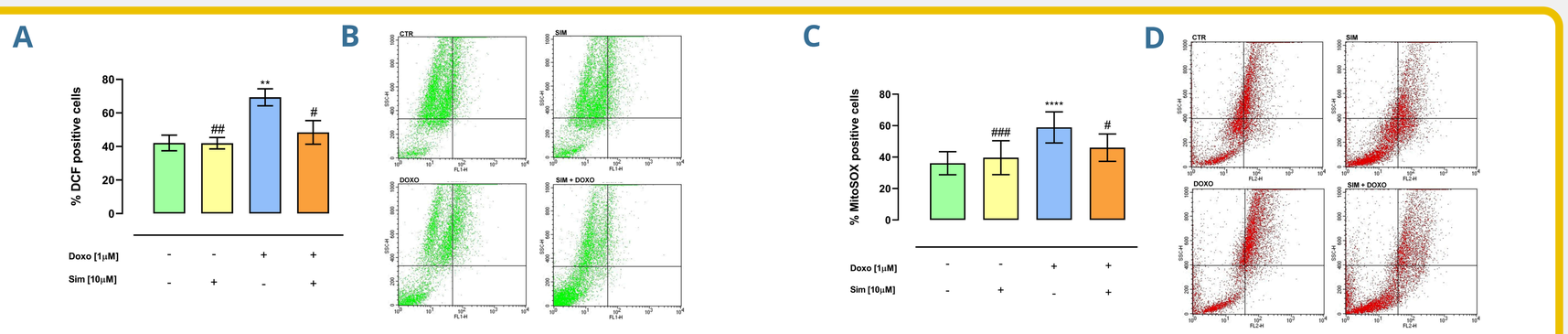


**Figure 2.** The fluorescent probes 2'-7'dichlorofluorescein diacetate (H2DCF-DA) and MitoSOX Red, a Mitochondrial Superoxide Indicator, were used to evaluate cytosolic ROS and mitochondrial superoxide generation, respectively. Data were analyzed by flow cytometry. Statistical analysis was performed using One-Way ANOVA followed by the Bonferroni multiple comparisons test. Values are expressed as mean ± SEM of the percentage of DCF and MitoSOX positive cells (n=3). ** p<0.01, **** p<0.001 vs untreated cells; # p<0.05, ## p< 0.01, ### p<0.005 vs Doxo-treated cells (A,C). Representative histograms for the flow cytometry analysis are reported in Panels (B,D).

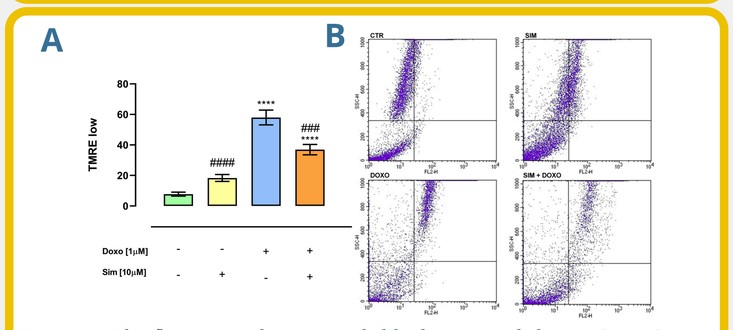


**Figure 3.** The fluorescent dye tetramethylrhodamine methyl ester (TMRE) was used to evaluate mitochondrial membrane potential. Data were analyzed by flow cytometry. Statistical analysis was performed using One-Way ANOVA followed by the Bonferroni multiple comparisons test. Values are expressed as mean ± SEM TMRE-positive cells percentage (n=3). **** p<0.001 vs untreated cells; ### p<0.005 and #### p< 0.001 vs Doxo-treated cells (A). Representative histograms for the flow cytometry analysis are reported in Panels (B).

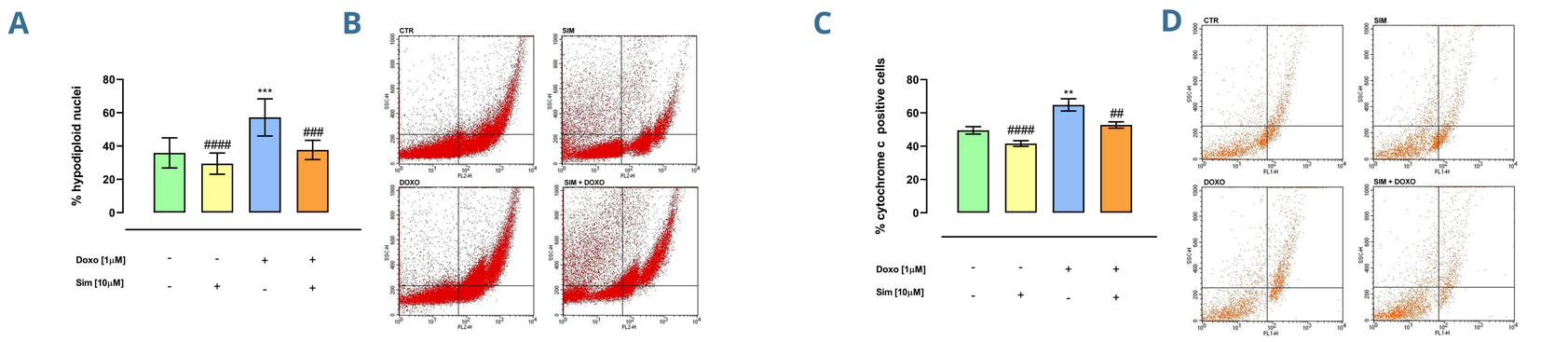


**Figure 4**. HCM were stained by propidium iodide and fluorescence of individual nuclei was measured by flow cytometry. To evaluate cytosolic cytochrome c levels flow cytometry analysis was used. Statistical analysis was performed using the One-Way ANOVA followed by the Bonferroni multiple comparisons test. Values are expressed as mean ± SEM of % hypodiploid nuclei and cytochrome c positive cells (n=3) (Panel A and C). ** p<0.01, *** p<0.005 vs untreated cells; ## p<0.01, ### p<0.005, #### p<0.001 vs Doxo-treated cells. Representative histograms for the flow cytometry analysis are reported in Panels (B, D)

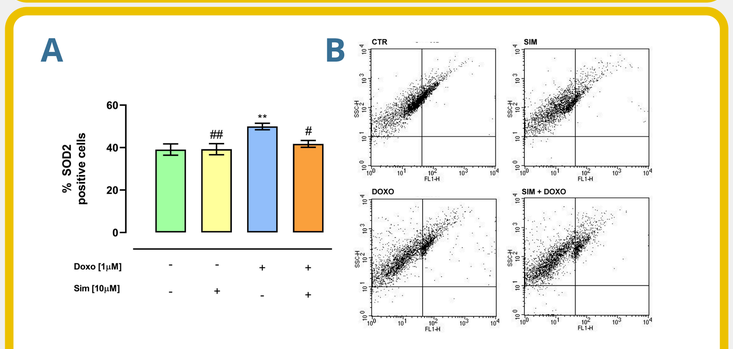


**Figure 5.** To evaluate SOD2 level flow cytometry analysis was used. Statistical analysis was performed using One-Way ANOVA followed by the Bonferroni multiple comparisons test. Values are expressed as mean ± SEM of % of SOD2 positive cells (n=3) (Panel A). ** p<0.01 vs untreated cells; # p<0.05 and ## p<0.01 vs Doxo-treated cells. Representative histograms for the flow cytometry analysis are reported in Panels (B).

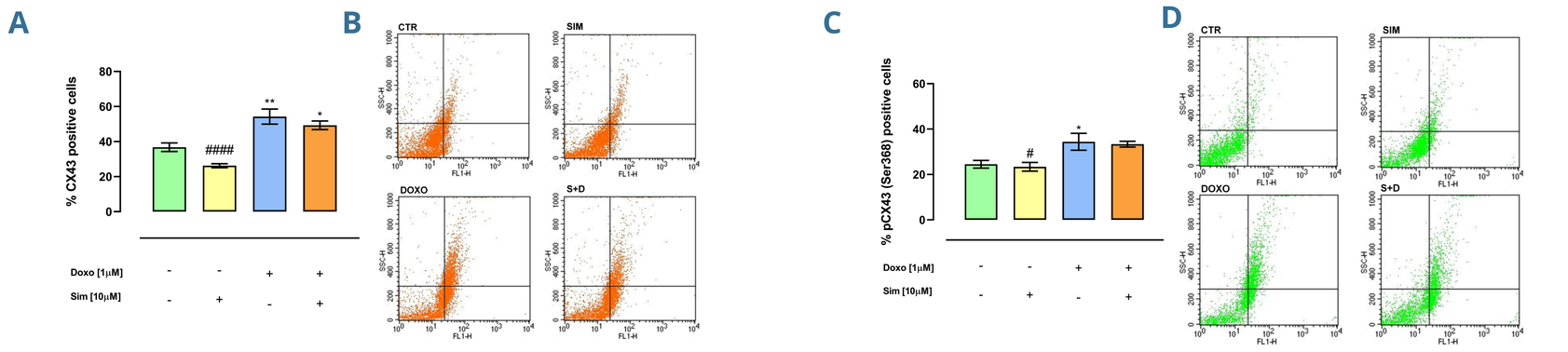


**Figure 6.** To evaluate membrane level of Cx43 and pCx43 (Ser368) flow cytometry analysis was used. Statistical analysis was performed using One-Way ANOVA followed by the Bonferroni multiple comparisons test. Values are expressed as mean ± SEM of % Cx43 or pCx43 (Ser368) positive cells (n=3) (Panel A and C). * p<0.05 ** p<0.01 vs untreated cells; # p<0.05, #### p<0.001 vs Doxo-treated cells. Representative histograms for the flow cytometry analysis are reported in Panels (B, D)

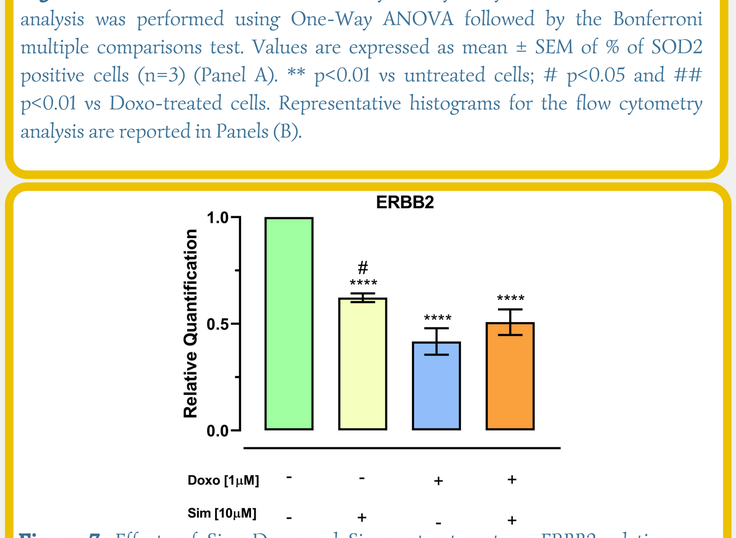


**Figure 7.** Effects of Sim, Doxo and Sim co-treatment on ERBB2 relative gene expression in a Human Cardiomyocyte cell line, as determined by real-time RT PCR. Data were calculated using the $2^{-\Delta\Delta Ct}$ method, normalized to GAPDH cDNA levels and then expressed as relative to control (calibrator sample, defined as 1.00). Data are expressed as means ± SD and were analyzed by analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. **** p<0.001 vs untreated cells; # p<0.05 vs Doxo-treated cells

## CONCLUSION

Simvastatin co-treatment, in our experimental model, was shown to alleviate oxidative stress and reduce apoptosis, thus leading human cardiomyocytes to lower their defense responses.
This indicates that Simvastatin could act as a potential therapeutic approach to prevent acute Doxorubicin-induced cardiotoxicity.

**References**
**1** Rawat P.S.; et al., Biomed Pharmacother. 2021, 139:111708 **2** Pecoraro, M.; et al., Toxicol Appl Pharmacol. 2016 293:44–52 **3** Renu, K.; et al., Eur. J. Pharmacol. 2018, 818, 241–253
**4** Kunutsor S.K.; et al., Cardiology. 2020;145(6):384-386. **5** Refaie, M., M.; et al., Human and Experimental Toxicology Volume 2022,41: 1–11 **6** Pecoraro, M.; et al., Int. J. Mol. Sci. 2023, 24, 7573
**7** Dewidar, S.A.; et al., Med Oncol 41, 7 2024

**Funded by the European Union - Next Generation EU. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Commission. Neither the European Union nor the European Commission can be held responsible for them. PNRR MUR M4 C2 Inv. 1.5 CUP B83C22003920001**